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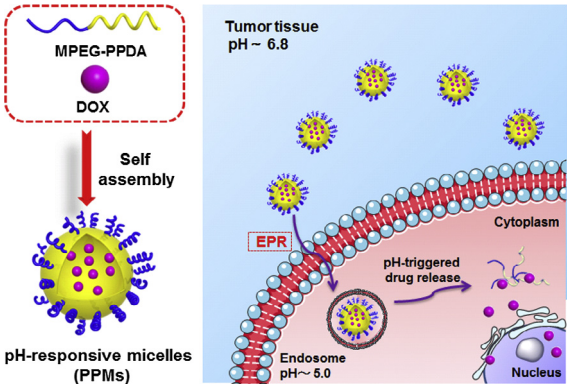
pH-responsive polymeric micelles based on poly(ethyleneglycol)-*b*-poly(2-(diisopropylamino) ethyl methacrylate) block copolymer for enhanced intracellular release of anticancer drugs

Zhigang Xu [a,*,1], Peng Xue [a,1], Yong-E Gao [a], Shiying Liu [b], Xiaoxiao Shi [a], Meili Hou [a], Yuejun Kang [a,*]

[a] Institute for Clean Energy and Advanced Materials, Faculty of Materials and Energy, Southwest University, Chongqing 400715, China
[b] School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459, Singapore

GRAPHICAL ABSTRACT



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ABSTRACT

We present a pH-responsive poly(ethyleneglycol)-*b*-poly(2-(diisopropylamino) ethyl methacrylate) block copolymer (MPEG-PPDA) that can self-assemble into micelles at very low critical micelle concentration. The formed micelles exhibit superior stability in physiological environment and pH-triggered transforming capability between self-assembly and disassembly. Moreover, the resulting micelles can load hydrophobic anticancer drug molecules such as doxorubicin in the core of micelles. The pH-triggered drug release kinetics matches the classical hydrazone bond model. The blank micelles demonstrate minimal cytotoxicity while the drug-loaded micelles exhibit significantly improved anticancer efficacy. These results indicate that this MPEG-PPDA block copolymer could be utilized as a universal pH-responsive delivery system for controlled release of hydrophobic anticancer drug in chemotherapy.

1. Introduction

In the past decade, polymeric drug delivery platforms have shown great potential in nanomedicine particularly for cancer therapy [1–10]. Generally, amphiphilic copolymers are capable of self-assembly into micelle nanoparticles and loading drug cargo in their hydrophobic cores, which can effectively adjust the pharmacokinetics and biodistribution of drug due to the enhanced permeability and retention (EPR) effect and thus dramatically improve cancer therapeutic efficacy [11,12]. Although various polymeric architectures including linear polymers [13–20], star-like polymers [21–23], hyperbranched polymers [24–26], cyclic polymers [27,28]

* Corresponding authors.
E-mail addresses: zgxu@swu.edu.cn (Z. Xu), yjkang@swu.edu.cn (Y. Kang).
[1] Equal contributors.

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have been used to construct the polymeric carriers for cancer therapy, only a few paradigms, such as SP1049C (doxorubicin-loaded micelle) and Genexol-PM (Paclitaxel-loaded micelle), have been approved for clinical applications or are currently subjected to clinical trials [29,30]. There are still some major challenges (e.g., uncontrolled drug release, low micellar stability, low drug efficacy and drug leakage) remained to be addressed prior to wide clinical applications of drug carriers based on polymeric micelles [31]. Therefore, it is critical to develop optimal polymeric drug systems that could resolve these major issues.

As a preferred polymeric architecture, linear polymers have many desirable features for nanomedicine, such as controllable structure and functionality, high micellar stability under normal physiological conditions, preferential accumulation at the target site based on passive or active targeting strategies, excellent biocompatibility and biodegradability [32,33]. Moreover, this architecture can be designed with a hydrophobic segment and a hydrophilic segment by self-assembly model. Recently, Zhang and co-workers described a type of biodegradable amphiphilic copolymers of poly (ethyleneglycol)-*b*-poly (ε-caprolactone) derivative for delivery of doxorubicin (DOX), which showed remarkable cytotoxicity against HepG2 tumour cells [34]. However, these architectures suffer from uncontrolled drug release and leakage. Compared to normal counterparts, tumour cells and tissues usually exhibit special characteristics, such as relatively low pH, high concentration of glutathione and other unique microenvironmental cues. As a rational approach, many researchers aim to design intelligent polymeric platforms taking advantage of these unique environmental cues for cancer therapy [17,35–41]. Notably, nanoplatforms based on pH-responsive polymers are able to target on the extracellular or intracellular pH condition of tumoral tissues and realize pH-triggered drug release, thereby reducing undesirable side effects while improving drug delivery efficiency. There are many existing strategies to tailor pH-responsive polymeric drug delivery platforms using acetal, *cis*-aconityl or Schiff base [42]. Meanwhile, these strategies are usually limited because of time consuming synthetic process, applicability solely to a particular drug model and non-specific cytotoxicity. For example, Sui and co-workers reported a pH-responsive DOX delivery system using a hydrazone bond and achieved high drug loading capacity and a pH-responsive controlled release [38]. However, the similar systems currently worked only with DOX model while had not yet able to realize the pH-triggered capability for other drug candidates.

In this work, we propose a pH-responsive polymeric carrier using an amphiphilic block copolymer poly(ethyleneglycol)-*b*-poly(2-(diisopropylamino) ethyl methacrylate), abbreviated as MPEG-PPDA, which has a few distinct features compared to other similar systems for drug delivery. Firstly, MPEG-PPDA polymer exhibits a relatively lower critical micelle concentration, which ensures the superior micellar stability of this system in normal physiological condition. Secondly, the resulting micelle shows pH-triggered transforming capability between self-assembly and disassembly, which is able to transport and release drug loads in the acidic endosomal microenvironment of tumour cells (Scheme 1). Thirdly, the hydrophobic DOX drug can be efficiently encapsulated into MPEG-PPDA micelles and internalized rapidly by tumour cells. The cytotoxicity and the anticancer efficacy of these drug-loaded micelles are investigated in vitro using HeLa cell model.

2. Experimental

2.1. Materials

All chemicals were acquired from Sigma-Aldrich (USA) except those indicated otherwise. MPEG (poly(ethylene glycol) methyl ether, Mn≈2000,) were dehydrated following a reported method [16]. BIBB (2-bromoisobutyryl bromide, 98%), TEA (triethylamine, 99%), PMDETA (N,N,N,N,N-pentamethyldiethylenetriamine, 99%), pyrene (98%), PDA (2-(diisopropylamino) ethyl methacrylate, 97%), CuBr (copper(I) bromide, 99%), Al_2O_3 (aluminium oxide), formalin solution and DAPI (4′,6-diamidino-2-phenylindole) were used as received. CuBr was further purified with acetic acid/methanol washing process three times. All anhydrous solvents including anhydrous propanol, THF (tetrahydrofuran) and DMF (N,N-Dimethylformamide) were used directly. Other organic solvents were provided by Sinopharm Chemical Reagent (China). DOX·HCl (doxorubicin hydrochloride) was acquired from HuaFeng United Technology (Beijing, China). FBS (fetal bovine serum), DMEM (Dulbecco's modified eagle's medium), penicillin/streptomycin mixture, PBS (phosphate buffered saline), TrypLE™ Express Enzyme, Alexa Fluor 633 phalloidin and cell viability reagent (PrestoBlue) was supplied by Life Technologies (Singapore). A water purification system (Milli-Q Synthesis A10, Molsheim, France) supplied the deionized (DI) water.

2.2. Characterization

^{1}H NMR spectra were measured with a Bruker AV 400 NMR (Rheinstetten, Germany) using $CDCl_3$ as solvent. Dynamic light scattering (DLS, BI-200SM, Brookhaven, USA) was used to characterize the size distribution and ζ-potentials of PPMs and PPMs-DOX with a detection angle of 90°. Transmission electron microscopy (TEM, JEM-1230EX, Japan) was used to characterize the morphology of the micelles. The TEM samples were prepared by adding a single drop of PPMs or PPMs-DOX solution on copper grids for measurement. A Fourier transform infrared (FTIR) spectrophotometer (Perkin Elmer, USA) was used to record the FTIR spectra and the pellet was obtained by potassium bromide (KBr). A gel permeation chromatography (GPC) system (Agilent 1260, USA) was used to measure the distribution (Mw/Mn) and the number-average (Mw) of molecular weight. We used THF as the eluent (1.0 mL min^{-1}) and polystyrene for standard calibration. A spectrophotometer (UV-2450, Shimadzu, Japan) was used to measure the ultraviolet and visible (UV–vis) spectra of the samples, and a fluorescence spectrometer (LS-55, Perkin Elmer, USA) was utilized to record the fluorescence spectra. The cell images were recorded using a confocal laser scanning microscope (LSM 780, Carl Zeiss, Germany).

2.3. Synthesis of MPEG-Br

The MPEG-Br polymer was synthesized as following: Briefly, MPEG (Mn: 2000, 4.0 g, 2 mmol) was dissolved in anhydrous THF (30 mL) in ice-water bath. Then TEA (10 mmol, 1.39 mL) and 2-bromoisobutyryl bromide (10 mmol, 0.62 mL) were introduced into the MPEG solution with magnetic stirring. After a 2 h reaction at 0 °C and further stirring under ambient temperature for about 22 h, the insoluble salts were removed by filtration and the THF solvent was concentrated in an evaporator. The resulting polymer was achieved using diethyl ether and dried under 40 °C in vacuum for 24 h to obtain a white powder of MPEG-Br (1.2 g, yield: 60%).

2.4. Synthesis of MPEG-PPDA

Typically, the block copolymer of MPEG-PPDA was prepared *via* atom transfer radical polymerization (ATRP) method, which used MPEG-Br as the macroinitiator of 2-(diisopropylamino) ethyl methacrylate (PDA). Briefly, MPEG-Br (420 mg, 0.2 mmol), PDA (2.15 g, 10 mmol) and CuBr (28.8 mg, 0.2 mmol) were dissolved in 3.0 mL DMF and 3.0 mL propanol in a 25 mL flask inside a glove box, and the mixture solution was degassed by three cycles of